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The Effects of Environmental Toxin Atrazine Exposure in *Danio rerio*: a Novel Pollutant that may promote the Pathogenesis of Alzheimer's Disease

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Abstract

Atrazine is one of the most prominent herbicides and environmental toxins in the world and is a member of the triazine class of herbicides. An estimated 82 million pounds of Atrazine is used in the US annually, and literature research suggests that the current wastewater treatment facilities are unable to efficiently remove it. Alzheimer's Disease (AD) is a neurodegenerative disease that is characterized by a buildup of toxic amyloid-beta plaques and tangles of hyperphosphorylated tau protein in the brain. Several key pro-and anti-Alzheimer's Disease genes have been shown to be involved in the pathogenesis of this disease, including AKT1s1, APOE, APPb, BACE1, BASP1, EPHA2, GSK3Aa, GSK3B, LEPR, NOTCH1a, SNCb, ARID5b, BIN1, CD2AP, COX1, MYO5Ab, PICALMa, PLD3, and TOMM40. Atrazine's effects on these known mediators of AD were analyzed using bioinformatics technology and analysis at 0 ppb, 0.3 ppb, 3.0 ppb, and 30 ppb (n=6). The gene expression profiles of the GSE72243 dataset were obtained from the Gene Expression Omnibus (GEO) database. Selected genes were then analyzed and graphed via Genespring 14.5 and Microsoft Excel. Significant dysregulation of the expression of both pro-and anti-Alzheimer's Disease genes was observed in the male Zebrafish brain. APPb, BACE1, EPHA2, GSK3B, LEPR, NOTCH1a, and SNCb were significantly upregulated, while ARID5b, BIN1, CD2AP, MYO5Ab, and PLD3 were significantly downregulated. Our results suggest that Atrazine promotes the buildup of toxic amyloid-beta plaques and tangles of tau proteins in male zebrafish brain cells. In sum, Atrazine appears to increase the risk of developing Alzheimer's Disease by increasing toxic amyloid-beta protein production and spread, increasing tau phosphorylation, allowing pathogens to enter the brain by weakening the blood-brain barrier (BBB), and weakening members of the peripheral blood lymphocyte (PBL) family that would otherwise suppress the development of AD.

Keywords: Atrazine; Alzheimer's Disease (AD); environmental toxins; herbicides; neurodegenerative disease; blood-brain barrier (BBB); water filtration.

Introduction

1.1 Rationale

Alzheimer's Disease (AD) is one of the most common neurodegenerative diseases among the elderly population (65 years and older) and is characterized by a buildup of toxic beta-amyloid plaques and tangles of hyperphosphorylated tau protein in the brain [36]. Those affected by AD experience memory loss, learning difficulties, and in severe cases, loss of bowel and bladder control [36]. AD currently affects 5.8 million people in the United States and costs families an estimated $290 billion in healthcare, hospice services, and long-term care for members in 2019 [4]. AD is ultimately fatal and was the sixth leading cause of death in the US in 2017 [4]. The development of AD can be attributed to a myriad of factors, including environmental and genetic [18, 22]. In addition, the upregulation of pro-Alzheimer's genes such as AKT1s1, APOE, APPb, BACE1, BASP1, EPHA2, GSK3Aa, GSK3B, LEPR, NOTCH1a, and SNCb as well as the downregulation of anti-Alzheimer's genes such as ARID5b, BIN1, CD2AP, COX1, MYO5Ab, PICALMa, PLD3, and TOMM40 have been shown to play a major role in the pathogenesis of AD [14, 16, 20, 22, 23, 31, 38, 43, 44, 46, 49, 52, 55-57].

1.2 Atrazine: Overview and Difficulties in Water Filtration

Atrazine (1-Chloro-3-ethylamino-5-isopropylamino-2,4,6-triazine) is a member of the triazine family and is one of the most prominent herbicides in the world with 82 million pounds being used in the US annually [13]. Atrazine is the active ingredient in many common pesticides, several of which are: AAtrex 4L, AAtrex Nine-0, Acuron, Beacon, and Envoke. Because of atrazine's low biodegradability and its wide use since the 1950s, it has been shown to extensively build up in the environment [29]. Atrazine is also shown to be stable in soil, resistant to hydrolysis, have a high vapor pressure, moderate water solubility, and high leakage potential, making it a potent surface and groundwater contaminant [33].

The United States Environmental Protection Agency (EPA) has set a Maximum Contaminant Level (MCL) of 3 ppb (3 micrograms per liter) for atrazine in water sources to try to minimize the effect of atrazine in the environment [10]. This amount, however, is often exceeded in drinking water supplies. In a study done by the EPA between 2003 and 2004, more than 90% of 139 water samples had detectable amounts of atrazine and 39% had peak levels of atrazine above the MCL [53]. Aside from drinking water sources, atrazine has also been reported to contaminate soft drinks, sports drinks, and wine [29, 50].

Studies have shown atrazine to be an endocrine disrupter and detrimental to the sexual health of organisms; causing congenital disabilities such as miscarriage and low birth weight in humans [28, 42]. Because of this, atrazine has been classified as both an environmental hazard and a health hazard by the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) as well as banned by the European Union [35, 42]. Although atrazine has been shown to be an endocrine disruptor, its effect on the pathogenesis of AD as well as other neurodegenerative diseases is relatively unknown.

A study done on the effects of atrazine on rats showed that 80% of a 0.53 mg dose was absorbed through the gastrointestinal tract into the bloodstream, and 15% was retained in body tissue [21]. This shows that atrazine is not completely removed from the body and will continue to build up as more is ingested. Another study done using in silico methods to predict intestinal absorption and brain penetration of pesticides in humans predicted triazine pesticides to be 100% permeant in the gastrointestinal tract and 36% permeant in the brain [12]. This suggests that atrazine is readily absorbed into the bloodstream through the gastrointestinal tract as well as able to pass through the blood-brain barrier.

The current standard filtration systems in the US cannot completely remove atrazine from drinking water. A study investigating the fate of endocrine-disruptor, pharmaceutical, and personal care product chemicals in drinking water treatment processes showed that atrazine had an overall low removal rate by common filtration processes [51]. Two processes that were relatively better at removing atrazine are absorption by Activated Carbon (AC) filters and nanofiltration by Reverse Osmosis filters [17, 24]. Reverse osmosis filters require more energy than the standard filters, however, making them more expensive to run and maintain [30]. AC filters are also expensive to regenerate and desorb substances

from, and periodic replacement of AC filters also adds to the operating costs [54]. It is because of these expenses and the reason these filters require more development that they are not frequently used or widespread in the US [7].

1.3 Danio rerio: A Model Organism

Zebrafish (*Danio rerio)* is regarded by scientists to be an exceptional model organism because of its small size, short generation time, fecundity, vertebrate physiology, and nervous system with many similarities to the mammalian central nervous system [2, 6, 40]. Zebrafish embryos also rapidly develop, are optically transparent, can be arranged into multiwell plates, and are permeable to a variety of small molecules and drugs [19]. At least 70% of the human genome has been shown to have an ortholog in zebrafish as well [25].

1.4 Purpose/Hypothesis

Purpose:

This study investigated the effects of atrazine at varying concentrations (0, 0.3 , 3, and 30 ppb) on AKT1s1, APOE, APPb, BACE1, BASP1, EPHA2, GSK3Aa, GSK3B, LEPR, NOTCH1a, SNCb, ARID5b, BIN1, CD2AP, COX1, MYO5Ab, PICALMa, PLD3, and TOMM40 gene expression profiles in male zebrafish brain cells.

Hypothesis:

It was hypothesized that Atrazine will upregulate the selected pro-Alzheimer's Disease gene expressions and downregulate the anti-Alzheimer's Disease gene expressions.

Methods

Data Acquisition:

The gene expression profiles of the GSE72243 dataset were obtained from the Gene Expression Omnibus (GEO) database. This particular dataset represented the gene expressions of male zebrafish brains at different concentrations of atrazine exposure (0 ppb, 0.3 ppb, 3 ppb, and 30 ppb). In addition, there existed six trials per variable and control.

Data Analysis:

Using Genespring 14.5 (Agilent Technologies, Santa Clara, CA), the gene expression levels of AKT1s1, APOE, APPb, BACE1, BASP1, EPHA2, GSK3Aa, GSK3B, LEPR, NOTCH1a, SNCb, ARID5b, BIN1, CD2AP, COX1, MYO5Ab, PICALMa, PLD3, and TOMM40 in the GSE72243 dataset were analyzed. Outliers were removed according to standard statistical procedures. The fold changes were measured by dividing the signals of the three variables by the control signal. The control signal represented the normalizing baseline for each of the other three variables. Any fold change greater than 2.0 and less than 0.5 was considered significant dysregulation. Fold change has also been used historically as the first method to identify changes in different gene expression levels, and a change of at least two-fold up or down has been considered meaningful in past studies [48]. Data was graphed via Microsoft Excel.

Results

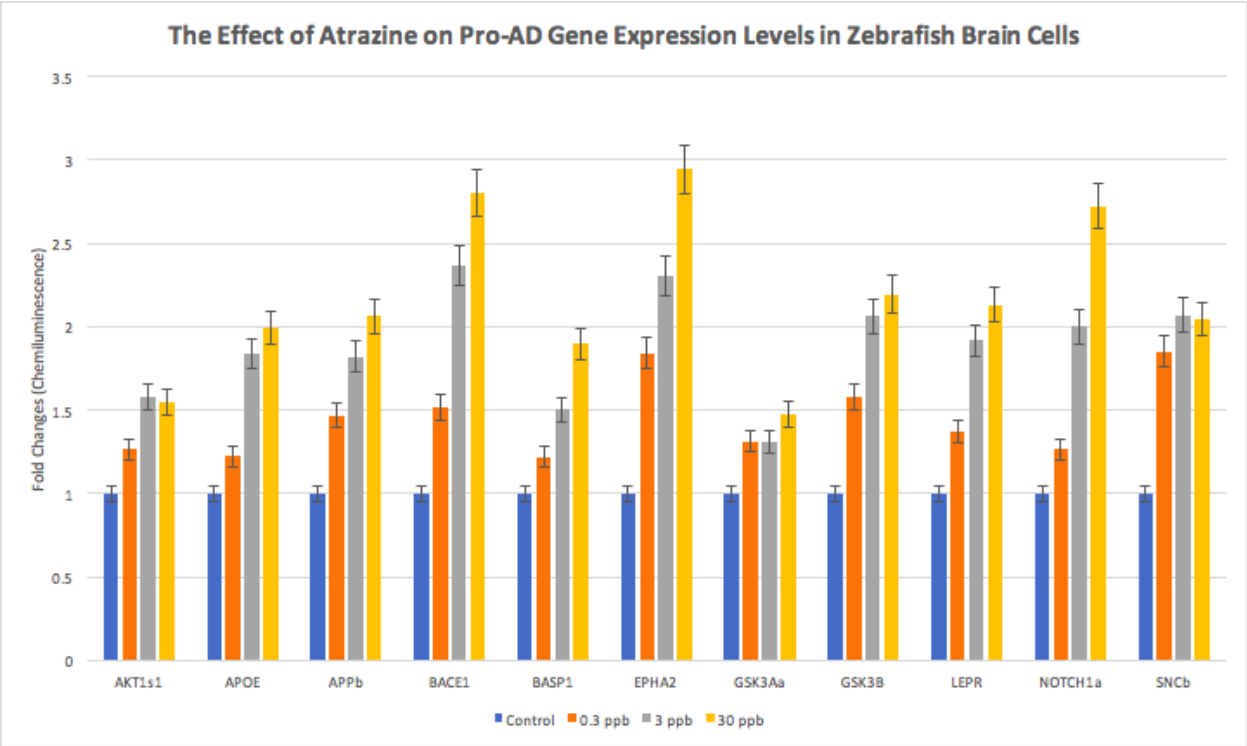

Figure 1: The x-axis of the graph represents the pro-AD genes: AKT1s1, APOE, APPb, BACE1, BASP1, EPHA2, GSK3Aa, GSK3B, LEPR, NOTCH1a, and SNCb. The y-axis represents the fold changes of the gene expression profiles in chemiluminescence when exposed to 0.3, 3, and 30 ppb (micrograms/liter) atrazine compared to the control (0 ppb atrazine). The pro-AD genes in zebrafish brain cells saw an overall increase in expression when exposed to various concentrations of atrazine. APPb expression increased 2.062 folds when exposed to 30 ppb atrazine. BACE1 expression increased 2.367 folds when exposed to 3 ppb atrazine and 2.798 folds when exposed to 30 ppb atrazine. EPHA2 expression increased 2.305 folds when exposed to 3 ppb atrazine and 2.942 folds when exposed to 30 ppb atrazine. GSK3B expression increased 2.064 folds when exposed to 3 ppb atrazine and 2.195 folds when exposed to 30 ppb atrazine. LEPR expression increased 2.132 folds when exposed to 30 ppb atrazine. NOTCH1a expression increased 2.723 folds when exposed to 30 ppb atrazine. SNCb increased 2.069 folds when exposed to 3 ppb atrazine and 2.046 folds when exposed to 30 ppb atrazine.

The pro-AD genes showed a general increase in expression when exposed to various concentrations (0, 0.3, 3, and 30 ppb) of atrazine (Figure 1). Genes that showed a significant change (fold change >2) were APPb, BACE1, EPHA2, GSK3B, LEPR, NOTCH1a, and SNCb. APPb expression increased 1.469 times when exposed to 0.3 ppb atrazine, 1.820 times when exposed to 3 ppb, and 2.062 times when exposed to 30 ppb. BACE1 expression increased 1.520 times when exposed to 0.3 ppb atrazine, 2.367 times when exposed to 3 ppb, and 2.798 when exposed to 30 ppb. EPHA2 expression increased 1.840 times when exposed to 0.3 ppb atrazine, 2.305 times when exposed to 3 ppb, and 2.942 times when exposed to 30 ppb. GSK3B expression increased 1.575 times when exposed to 0.3 ppb atrazine, 2.064 times when exposed to 3 ppb, and 2.195 times when exposed to 30 ppb. LEPR expression increased 1.368 times when exposed to 0.3 ppb atrazine, 1.916 times when exposed to 3 ppb, and 2.132 times when exposed to 30 ppb. NOTCH1a expression increased 1.263 times when exposed to 0.3 ppb atrazine, 1.998 times when exposed to 3 ppb, and 2.723 times when exposed to 30 ppb. SNCb expression increased 1.853 times when exposed to 0.3 ppb atrazine, 2.069 times when exposed to 3 ppb, and 2.046 times when exposed to 30 ppb.

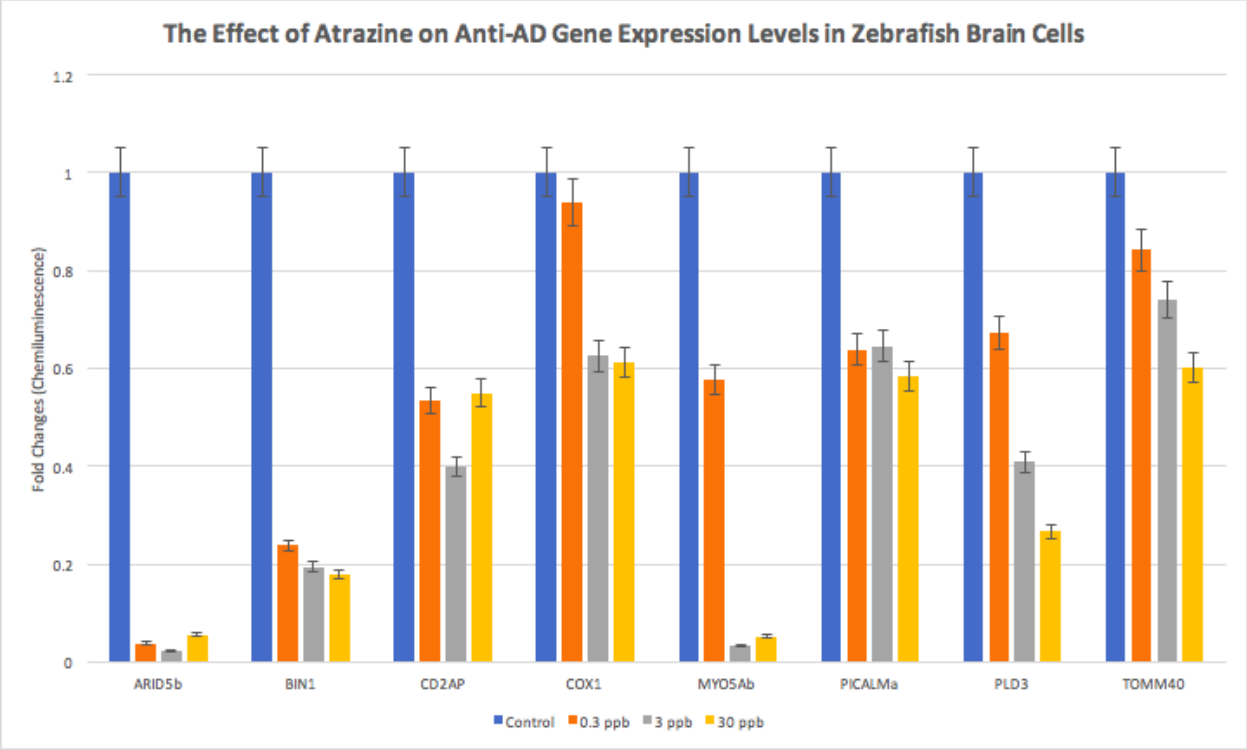

Figure 2: The x-axis of the graph represents the anti-AD genes: ARID5b, BIN1, CD2AP, COX1, MYO5Ab, PICALMa, PLD3, and TOMM40. The y-axis represents the fold changes of the gene expression profiles in chemiluminescence when exposed to 0.3, 3, and 30 ppb (micrograms/liter) atrazine compared to the control (0 ppb atrazine). The anti-AD genes in zebrafish brain cells saw an overall decrease in expression when exposed to various concentrations of atrazine. ARID5b expression decreased 0.039 folds when exposed to 0.3 ppb atrazine, 0.024 folds when exposed to 3 ppb atrazine, and 0.056 folds when exposed to 30 ppb atrazine. BIN1 expression decreased 0.238 folds when exposed to 0.3 ppb atrazine, 0.194 folds when exposed to 3 ppb atrazine, and 0.180 folds when exposed to 30 ppb atrazine. CD2AP expression decreased 0.400 folds when exposed to 3 ppb atrazine. MYO5Ab expression decreased 0.034 folds when exposed to 3 ppb atrazine 0.053 folds when exposed to 30 ppb atrazine. PLD3 expression decreased 0.408 folds when exposed to 3 ppb atrazine and 0.266 folds when exposed to 30 ppb atrazine.

The anti-AD genes showed a general decrease in expression when exposed to various concentrations of atrazine (Figure 2). Genes that showed a significant change (Fold change <0.5) were ARID5b, BIN1, CD2AP, MYO5Ab, and PLD3. ARID5b expression decreased 0.039 folds when exposed to 0.3 ppb atrazine, 0.024 folds when exposed to 3 ppb, and 0.056 folds at 30 ppb. BIN1 decreased 0.238 folds when exposed to 0.3 ppb atrazine, 0.194 folds when exposed to 3 ppb, and 0.180 folds at 30 ppb. CD2AP expression decreased 0.534 folds when exposed to 0.3 ppb atrazine, 0.400 folds when exposed to 3 ppb, and 0.549 folds at 30 ppb. MYO5Ab expression decreased 0.577 folds when exposed to 0.3 ppb atrazine, 0.034 folds when exposed to 3 ppb, and 0.053 folds at 30 ppb. PLD3 expression decreased 0.672 folds when exposed to 0.3 ppb atrazine, 0.408 folds when exposed to 3 ppb, and 0.266 at 30 ppb.

Conclusion

The purpose of this study was to investigate the effects of atrazine at varying concentrations on these genes that have been shown to support the development of AD: AKT1s1, APOE, APPb, BACE1, BASP1, EPHA2, GSK3Aa, GSK3B, LEPR, NOTCH1a, and SNCb, as well as these genes that have been shown by literature research to promote the development of AD when downregulated: ARID5b, BIN1, CD2AP, COX1, MYO5Ab, PICALMa, PLD3, and TOMM40. The data collected from the GSE72243 dataset suggested that APPb, BACE1, EPHA2, GSK3B, LEPR, NOTCH1a, and SNCb had significant

increases in gene expression profiles (>2 fold increase) when exposed to atrazine, while ARID5b, BIN1, CD2AP, MYO5Ab, and PLD3 had significant decreases in gene expression profiles (<0.5 fold decrease).

The Swedish APP mutation is a double point mutation at the amino-terminus of the AB domain neighboring the B-secretase site where lysine and methionine are replaced by asparagine and leucine [22]. This mutation causes an increase of irregular cleavage of APP by BACE, which leads to tau protein hyperphosphorylation and consequently AD [45]. Another APP mutation that has also been shown to lead to the manifestation of AD is the London mutation. The London mutation is a mutation at the C-terminus of the AB domain which alters gamma-secretase function and increases AB42 expression and lowers AB40 expression [22]. These previous studies on various mutations of APP suggest that the upregulation of the APP gene may lead to the formation of AD.

BACE1 has also been shown to play an important role in the formation of AD by catalyzing the initial cleavage at the B-site of APP. Amino acid differences caused by genetic changes at the B-cleavage site of APP may cause increased BACE1 activity toward the APP substrate and is associated with AD [27].

Relevant research suggests that EPHA2 plays a role in the permeation of the blood-brain barrier as well as AD development. Studies done in the past suggest that AD may be caused by fungal infections in the brain [41]. A study that treated brain endothelial cells with an EPHA2 chemical agonist saw greater migration of fungal cells across the BBB, indicating that upregulation of EPHA2 may increase the likeliness of the formation of AD by allowing pathogens to pass through the BBB [1].

Hyperactive GSK3B causes tau phosphorylation in AD making it so that the upregulation of this gene greatly increases the chances of the development of AD [44]. Relevant research also found that GSK3B was upregulated in neurons derived from AD-induced pluripotent stem cells, making GSK3B upregulation highly associated with AD [37].

Leptin is a hormone that suppresses energy intake and stimulates energy expenditure.
A study done on leptin-resistant db mutation mice that exhibit a lack of leptin signaling due to a point mutation in the leptin receptor (LEPR) showed that they had improved memory and lower amounts of AB plaques, suggesting lowered LEPR functionality's role in the clearance of toxic AB plaques in AD [56].

Accumulation of Aβ in down syndrome brain is thought to result from enhanced processing and overexpression of APP, which is thought to be because of a triplication of the 21 chromosome where the APP gene resides. Literature research suggests that down syndrome fibroblasts and AD cortex have been shown to overexpress NOTCH1 and Dll1, indicating that increased Aβ production and overexpression of NOTCH1 may lead to the neurodegeneration exhibited in both AD and down syndrome [52].

A study done on alpha-, beta-, and gamma-synuclein quantification in cerebrospinal fluid showed that βSyn (SNCb) and γSyn are increased in AD and Creutzfeldt-Jakob Disease (CJD) [38]. The study even went on to suggest SNCb as an alternative biomarker to or in combination with tau because of the strong correlation of SNCb with tau protein, indicating increased SNCb expression' role in the pathogenesis of AD.

After being identified as an AD-associated gene, ARID5B's involvement in the entorhinal cortex thickness and volume in cognitively healthy individuals was identified [49]. The entorhinal cortex has also been shown by past research to be one of the most severely damaged areas by AD as well as an area where neurodegenerative changes made by AD are likely to begin, making the downregulation of ARID5B a possible factor in the initial formation of AD [24, 49].

BIN1 has been identified as the second most prevalent risk factor in AD with downregulated levels in AD diseased brains [11]. Previous research suggests that lower levels of BIN1 promote the spread of tau pathology by increasing tau aggregate internalization through endocytosis and endosomal trafficking. This is because BIN1 reduces tau pathology propagation by negatively regulating endocytic flux and blocking endocytosis by inhibiting dynamin, a large GTPase that aids in the detachment of vesicles during endocytosis [11].

CD2AP has been shown to be involved in dynamic actin remodeling and membrane trafficking during endocytosis and cytokinesis [47]. Relevant research suggests that the progression from mild cognitive impairment to AD may be linked to a decreased suppressive ability in regulatory T cells, which

are a part of the peripheral blood lymphocyte (PBL) family [9]. A study done on the CD2AP saw that CD2AP was severely underexpressed in the PBLs of patients with sporadic AD which may have caused a decrease in PBLs [47]. Another study reported that underexpressed CD2AP in the endothelial cells adjacent to the brains of mice would severely weaken the BBB, which left the mice susceptible to seizures and other forms of disease [14]. The weakening of PBLs and the BBB suggests CD2AP underexpression may play a role in the manifestation of AD.

According to literature research, kinesin 5 (Eg5) has been shown to play an important role in the transport of neurotrophin and neurotransmitter receptors in neurons, and is severely underexpressed in AD patients, leading to a rapid decline in memory [5]. Another study suggested Myosin V (from the MYO5 gene) and Kinesin enhance each others' processivity and proposed that each motor acted as a tether for the other, preventing diffusion away from the microtubule track, allowing more steps to be taken before dissociation [3]. This suggests that decreased expression of both the various forms of Myosin V (MYOVa, MYOVb, MYOVc) and Kinesin may decrease the rate at which neurotrophin and neurotransmitter receptors are transported, leading to memory decline and accelerated progression of AD.

Recent studies show that PLD3 is very highly expressed in areas that are susceptible to AD (frontal, temporal, and occipital cortices as well as the hippocampus) in the brains of healthy individuals and severely underexpressed in those with AD [8]. Another study stated that PLD3 influenced APP processing by acting as a negative regulator because PLD3 overexpression in cultured neuroblastoma cells correlated with lower intracellular APP, suggesting PLD3's underexpression to play a role in increased APP expression [15, 22].

At various concentrations (0, 0.3, 3, and 30 ppb) of atrazine exposure, the gene expression profiles of several genes that have been shown by literature research to support the development AD when upregulated (APPb, BACE1, EPHA2, GSK3B, LEPR, NOTCH1a, and SNCb) were significantly increased (>2 folds) in male zebrafish brain cells. Through various pathways, the upregulation of these genes has been shown by previous studies to increase toxic AB production, increase tau phosphorylation, and allow pathogens to enter the brain by weakening the BBB, all of which potentially allow for the accelerated development of AD. At the aforementioned concentrations of atrazine exposure, the gene expression profiles of several genes that have been shown to promote the formation of AD when downregulated (ARID5b, BIN1, CD2AP, MYO5Ab, and PLD3) were significantly decreased (<0.5 folds). Through various pathways, the downregulation of these genes has been shown to weaken areas of the brain where AD is likely to develop, weaken members of the PBL family that would otherwise suppress the development of AD, allow for the spread of AB plaques in the brain, and accelerate rapid memory decline, which may all support the formation of AD. Overall, data from this study suggests the popular herbicide atrazine may be a factor that contributes to the pathogenesis of Alzheimer's Disease.

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